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Lectins Histochemical Studies in Submandibular Salivary Gland of the House Musk Shrew, *Suncus murinus*

Apinun Suprasert, Sahathep Juntaravimol, Pibul Ruensupapichat, Dollada Srisai and Seri Koonjaenak

ABSTRACT

Submandibular glands of male house musk shrew, *Suncus murinus*, were examined by light microscopic histochemical methods. The staining procedures employed were horseradish peroxidase conjugated lectins, Alcian blue (AB) pH 1.0, AB pH 2.5, Periodic acid-Schiff (PAS) and AB pH2.5-PAS in combination with enzyme digestion with neuraminidase. The lectins used in the present study were Peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), Wheat germ agglutinin (WGA), *Limax flavus* agglutinin (LFA), *Ulex europaeus* agglutinin-I (UEA-I), and *Loutus tetragonolobus* agglutinin (LTA)

The submandibular gland of *Suncus murinus* is a branched tubuloacinar gland. Its secretory endpiece contains both serous and mucous cells. Granular ducts, modified striated ducts, were found to be well developed. All the mucous acinar cells were colored deep blue with the AB pH 2.5-PAS procedure and were stained strongly with LFA. Neuraminidase digestion changed the deep blue coloration with the AB pH 2.5-PAS procedure to light red and abolished all staining of mucous cells with LFA. Removal of sialic acid with neuraminidase imparted weak to strong affinity for PNA. Serous cells showed strong red coloration with the AB pH2.5-PAS procedure and were reacted strongly with PNA, DBA and WGA. Granular duct cells exhibited moderate reaction with PAS, LTA and UEA-I.

Key words: lectin, submandibular, salivary gland, house musk shrew

INTRODUCTION

House musk shrew, *Suncus murinus*, a mammalian species belonged to Soricidae in Insectivora is generally regarded as having close evolutionary affinity with fossil primate. These mammalian species has recently been domesticated as a new experimental animal (Oda and Kondo, 1977; Kondo *et al*., 1978). Although a large number of papers have been published on various organs of the house musk shrew (Cooper and Bedford, 1976; Dryden and Anderson, 1977), our knowledge of morphology and histochemistry of the submandibular gland of this animal is still very limited.

The glycoconjugates in secretory epithelium of mammalian submandibular gland have been previously studied (Shackleford and Klapper, 1962; Pinkstaff, 1975; Menghi *et al*., 1983). Furthermore, an information has been obtained regarding the cytochemistry of glycoconjugates in the comparable mandibular gland of the chicken (Suprasert *et al*., 1986). The glycoconjugates are found in intracellular as well as extracellular sites in most, if not all, tissue. The significance of the glycoconjugates is poorly understood but they have been implicated in

Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900 Thailand.

a wide range of important biological activity such as cell adhesion and recognition, fertilization, growth, differentiation, and also in many pathological processes, including malignancy. (Sharon and Lis, 1982).

In view of the circumstance mentioned above, it is the aim of this study to investigate the morphology and the distribution of the glycoconjugates in secretory epithelium of the submandibular of the house musk shrew by means of lectins, a current available light microscopic methods.

MATERIALS AND METHODS

A total of 12 adult male musk shrews were the donors of the submandibular gland examined. After the animals were sacrificed by exsanguination under ether anesthesia their submandibular glands were dissected out. Tissue pieces from these glands were fixed in one of the following fixative 1) 10% formalin containing 2% calcium acetate for 12-24 h. at 4°C. The tissue specimens were then dehydrated in graded ethanol series, cleared with benzene and embedded in paraplast.

Sections were cut at a thickness of 3µm and then subjected to the following histological and histochemical staining procedures.

1. Hematoxylin-eosin (HE) procedure for general structures.

2. AB pH 1.0 procedure (Lev and Spicer, 1964) for sulfated glycoconjugates.

3. AB pH 2.5 procedure (Spicer *et al*., 1967) for acidic glycoconjugates.

4. Periodic acid-Schiff (PAS) (Pearse, 1968) for vicinal diol groups of glycoconjugates.

5. AB pH 2.5-PAS (Spicer *et al*., 1967) for differentiating acidic and neutral glycoconjugates.

Lectin staining procedures

To access the saccharides residues further, the peroxidase conjugated lectin diaminobenzidine procedure was performed to the paraplast sections. Following lectin were employed : *Limax flavus* agglutinin (LFA), *Peanut agglutinin* (PNA), wheat germ agglutinin (WGA), *Dolichos biflorus* agglutinin (DBA)

Furthermore, the following confirmation and control experiments were performed as well.

1) *Enzyme digestion:* Neuraminidase (form *Arthrobacter ureafaciens*). Prior to stain with AB pH 2.5, AB pH 2.5-PAS, LFA, and PNA, sections were incubated in 0.1 M acetate buffer (pH 5.3) containing 1 unit/ml of the enzyme and 0.04 M $CaC1_2$ at 39-41°C for 12-16 h. (Spicer *et al*., 1967)

For the enzyme digestion procedures, two types of control procedures were performed: a) some sections were incubated in respective buffer solutions without enzymes under the identical conditions of temperature and duration, b) The others sections were kept intact without any incubation procedures.

RESULTS

The submandibular gland of *Suncus murinus* is a branched tubuloacinar gland. An adenomere of the submandibular gland has secretory portion composed of glandular cells (secretory endpieces), conducting intercalated ducts, and granular duct cells (modified striated duct cells). Granular duct cells are well developed. At the base of secretory endpieces, myoepithelial cells are present. The secretory endpieces consisted of two layer of cuboidal cells of varying heights. The cuboidal cells are uninucleate and provided with secretory granules between nucleus and free surface. In the space between adenomere, relatively small amounts of connective tissue elements are interposed.

When tissue sections were reacted with AB pH 1.0, the cytoplasm of all secretory cells was found to exhibit negative reaction. The AB pH 2.5, likewise, to divide the secretory endpiece into two types : cells either with a strong alcianophilic cytoplasm at outer layer (mucous cells) or with a negatively AB pH 2.5 reactive cytoplasm at inner

layer (serous cells). When the secretory endpieces were reacted with PAS, the mucous cells at the outer layer were weakly positive. In contrast, the serous cells at inner layer were strongly positive. In the secretory endpieces of the submandibular gland, the dual staining with AB pH 2.5-PAS (Figure 1) resulted in deep blue coloration for the mucous cells at the outer layer and in deep red coloration for the serous cells at the inner layer.

The striated duct cells and granular duct cells were stained negatively with AB pH 1.0, AB pH 2.5. However, they were stained moderately with PAS. The mast cells of connective tissue were found to exhibit strong positive reaction with AB pH 1.0. However, they stained negatively with AB pH 2.5 and PAS.

Digestion with neuraminidase greatly diminished the intensity of AB pH 2.5 reaction the outer layer of the secretory endpieces. Neuraminidase digestion changed the deep blue coloration with the AB pH 2.5-PAS procedure to light red for the mucous cells (Figure 2)

When the secretory endpieces were reacted with lectins, mucous cells were strongly and moderately positive with LFA (Figure 3) and PNA (Figure 4) repectively. In contrast serous cells were negatively stained with LFA (Figure 3) and strongly positive with PNA (Figure 4) and WGA. The serous cells were furthermore found to be negative with UEA-I and LTA but the mucous cells were weakly positive with UEA-I and LTA. The granular duct cells were stained negatively with PNA, LFA and WGA. However they were stained moderately with LTA and UEA-I.

After neuraminidase treatment, the mucous cells stained strongly with PNA. In contrast, LFA did not stain mucous cells after the treatment. In the control tissues for the enzyme digestion experiments, the results of staining reactions were nearly comparable in intensity to those observed in sections kept intact without any incubation procedures.

All the results obtained are also summarized in Table 1.

DISCUSSION

The morphological, histochemical and biochemical characteristics of the mammalian salivary glands have been the objective of numerous researches (Leeson, 1967 ; Shackleford and Klapper, 1962; Bondi *et al*., 1978). A marked diversity of these characteristics have been noted by many investigations, including Shackleford and Klapper (1962) and Leppi and Spicer (1966). Such studies are chiefly abundant in rodents, because of the characteristic features of the gland : 1) the homogenous seromucous or serous cells of the secretory endpieces. 2) the presence of granular convoluted tubule cells, and 3) the sexual dimorphism of the gland. However, no previous publication was found on the morphology and histochemistry of the musk shrew submandibular gland.

In view of the staining specificities of AB pH 2.5 (Spicer *et al*., 1967) and PAS (Pearse, 1968). Two cell types were identified histochemically in the secretory endpieces of the musk shrew submandibular gland : neutral glycoconjugates-containing cells (serous cells) that react strongly with PAS, and acidic glycoconjugates containing cells (mucous cells) that react strongly with AB pH 2.5. The mucous cells were also contained some amount of neutral glycoconjugates as judged from deep blue coloration when they stained with AB pH 2.5-PAS (Spicer *et al*., 1967). In general, the serous cells were found at inner layer of the secretory endpieces and the mucous cells existed at the outer layer. In the light of the substrate specificity of neuraminidase (Spicer *et al*., 1967), Furthermore, the presence effects of digestion of this enzyme upon the AB pH 2.5 and AB pH 2.5-PAS reaction of the mucous cells are taken to indicate the existence of the sialic acid residues.

In view of the positive WGA and DBA reaction of the mucous cells, the glycoconjugates furthermore contained a notable amount of N-acetylglucosamine and N-acetygalactosamine. The

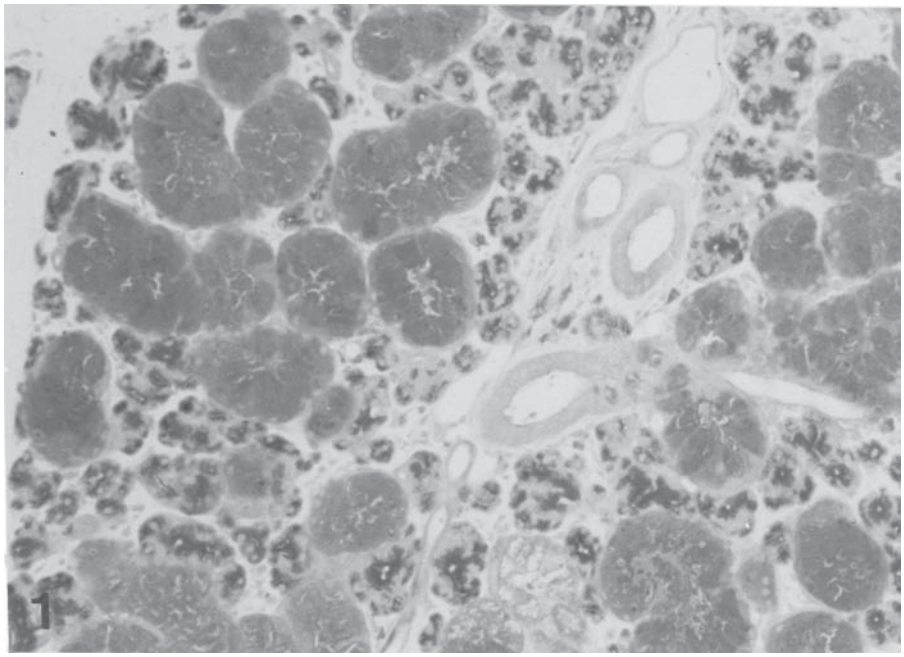

Figure 1 The secretory endpieces of musk shrew submandibular gland consist of serous and mucous cells. The dual staining with AB pH2.5-PAS resulted in deep blue with mucous cells and strong red with serous cells. The granular duct cells are well developed and stained moderately with PAS. X 120.

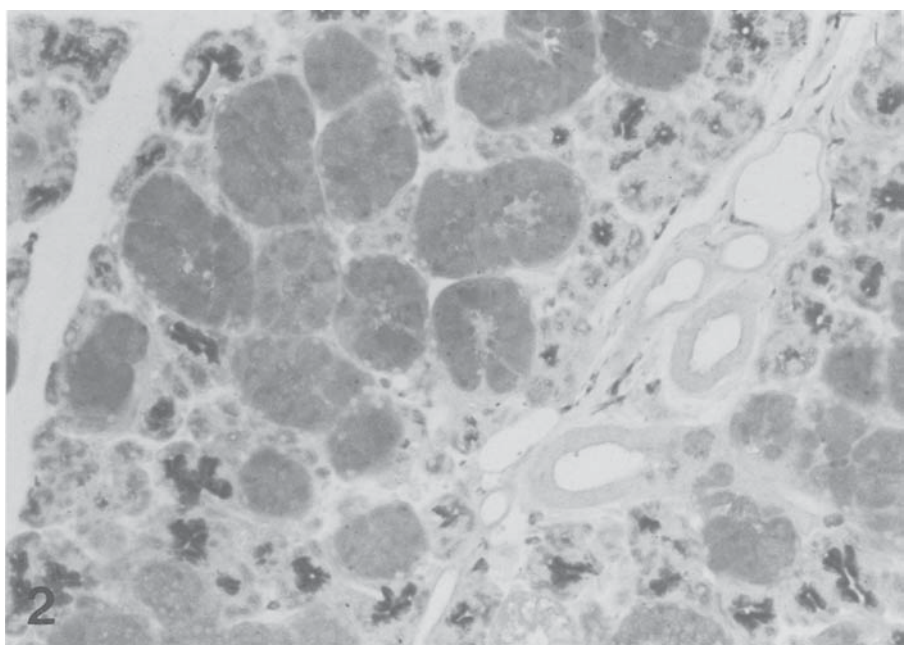

Figure 2 Neuraminidase digestion changed the deep blue coloration with AB pH 2.5-PAS procedure to light red for the mucous cells. X 120.

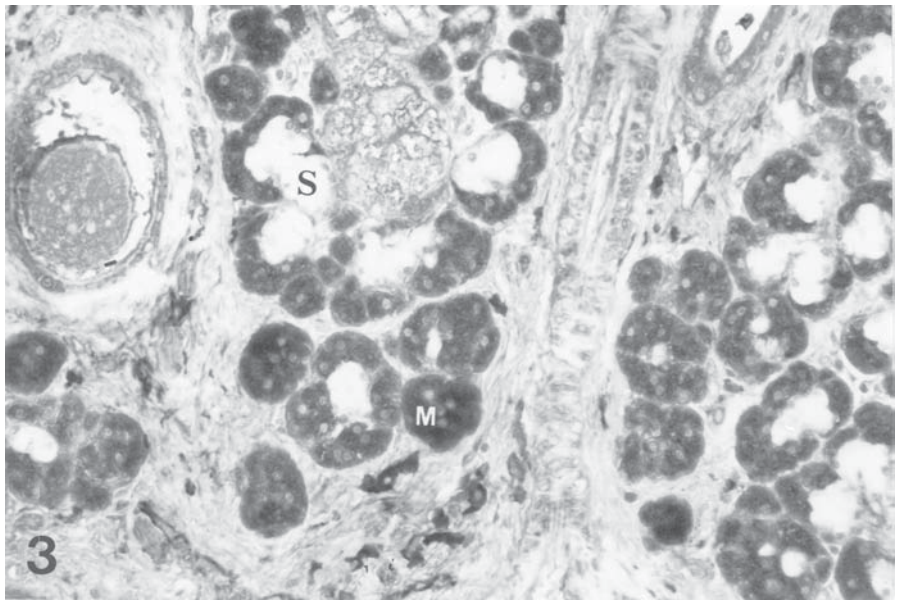

Figure 3 The mucous cells (M) stained strongly with LFA. In contrast, the serous cells (S) stained negatively with LFA. X 264

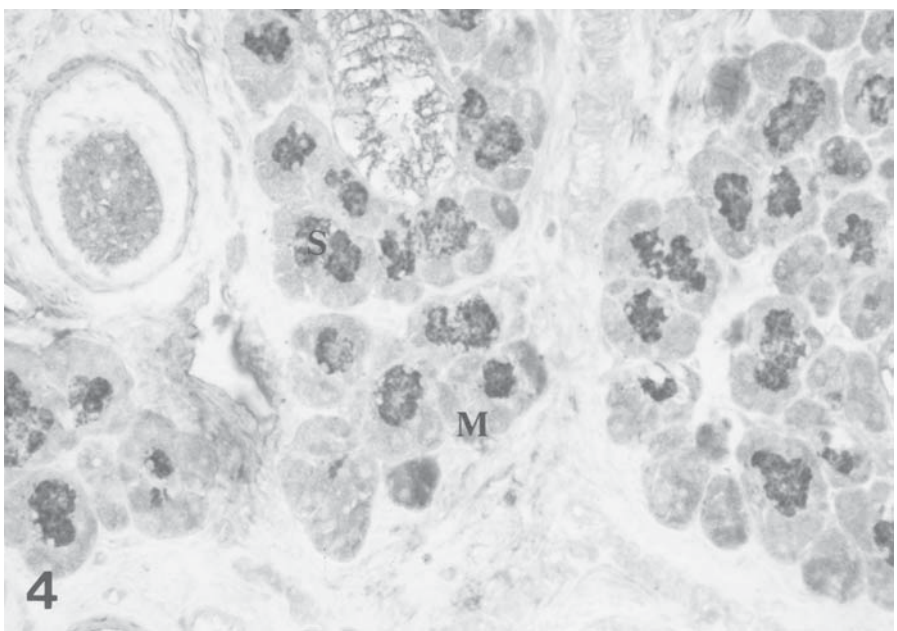

Figure 4 The serous cells (S) exhibit strong reaction with PNA. In contrast, the mucous cells (M) exhibited moderately reaction with PNA. X 264.

Table 1 Histochemical reaction of glycoconjugates in submandibular gland of the house musk shrew.

Staining procedures \ Histologic structures	Serous cells	Mucous cells	Granular duct cells
AB pH 1.0	0	0	0
AB pH 2.5	0	3 B	0
PAS	4 M	1 M	2 M
AB pH 2.5 – PAS	4 M	3 MB	2 M
N.AB pH 2.5	0	0	0
N.AB pH 2.5 – PAS	3-4 M	1-2 M	2M
LFA	0	4 Br	0
PNA	4 Br	1-2 Br	0
WGA	2 Br	1 Br	0
DBA	3 Br	1 Br	0
UEA-I	0	1 Br	2 Br
LTA	0	1 Br	1–2 Br
N.LFA	0	0	0
N.PNA	1 Br	3 Br	0-1 Br

Abbreviation
B = Blue, Br = Brown, M = Magenta, O =Negative reaction
1-n = number indicates intensity of staining reaction
N = neuraminidase

positive staining of PNA at serous cell and LFA at mucous cells suggested that terminal dimer galactose-(1-3) N-acetylgalactosamine occur in secretery granules of serous cells, while terminal sialic acid residues occur in granules of mucous cells. The presences of sialic acid-galactose dimer in mucous cells were more confirmed since an enhanced PNA reaction could be detected following digestion with neuraminidase.

The histochemical study in present investigation permits the identification of differences between elements of the secretory endpieces and those that granular duct cells. As a matter of fact, while the former contains primarily acid component in the mucous cells and neutral component in the serous cells, in the latter involves on neutral glycoproteins.

All the tissue structures of the musk shrew submandibular gland are found to devoid of sulfated groups as presummed from negative reaction with AB pH 1.0 (Lev and Spicer, 1964). This is in contrast to the acini in submandibular gland of various mammalians and avian which contain sulfomucins (Bondi *et al*., 1984, Suprasert *et al*., 1986).

The results of the present study with regard to the morphology and histochemistry have some controversy surrounds the cellular composition of the submandibular gland. In the musk shrew, the secretory endpiece is, of course, serous and mucous cells. In contrast, the submandibular gland of most rodents presents a homogenous secreting components which exhibit morphologically a single serous type but histochemically they display seromucous characteristics (Shackleford and Klapper, 1962). Furthermore, the comparable mandibular glands of the chicken are found to contain exclusively with mucous cells (Suprasert *et*

al., 1986). However, the present of granular duct cells in submandibular gland of musk shrew is similar to those of the rodents but is contrast to those of the chickens.

It is special interesting that at least two types of cells were histochemically differentiated in the secretory endpieces and one cell type in the granular convoluted tubule of the musk shrew submandibular gland examined in the present work. This fact appears to support to the concept that the secretory glycoconjugates are various types in chemical nature and that the physiological activities performed by this carbohydrates are therefore of diversified feature.

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